

# Gene Jockeys Fight *Fusarium* Head Blight

JACK DYKINGA (K5147-3)



Geneticist Ann Blechl and colleagues are the first to insert modified *Fusarium* chitinase and glucanase genes into wheat plants, which may lead to wheats that are more resistant to *Fusarium* head blight.

BRUCE FRITZ (K8472-2)



Lighter, discolored barley heads are infected with *Fusarium* head scab fungi.

In some years—no one can predict precisely which—young wheat and barley plants are beleaguered by a formidable fungal foe known as *Fusarium graminearum*. It is responsible for a disease known as wheat scab, or *Fusarium* head blight, which causes plump kernels to shrivel and take on an unhealthy, bleached, scabby appearance.

Right now, there’s no effective control for this plant disease. Losses in any given year will vary, but can be enormous. From 1998 to 2000, for example, *Fusarium*-related losses amounted to an estimated \$2.7 billion in the north-central and Great Plains states.

ARS, industry, and university scientists have joined forces to equip tomorrow’s wheat and barley plants with a new weapon: genes that might provide a stronger natural defense against this longtime enemy.

The researchers are constructing some of these genes with pieces of genetic material from *Fusarium* itself. This “dirty trick” strategy may deceive the fungus and sidestep its natural defenses. What’s

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more, this work might be applicable for fighting other major crop pests.

That's the plan of ARS geneticists Patricia A. Okubara at Pullman, Washington, and Ann E. Blechl at Albany, California. They're collaborating with Thomas M. Hohn, formerly with ARS at Peoria, Illinois, and now with Syngenta at Research Triangle Park, North Carolina, and Randy M. Berka of Novo Nordisk, in Davis, California. The scientists are seeking a patent for some of their innovative, antifungal genes.

#### Designer Genes May Quell Fungus

The researchers designed genes to cue wheat plants to make enzymes called chitinase and glucanase. These are the types of enzymes *Fusarium* manufactures when it needs to tear down some of its cell walls to grow and expand. Cell walls of *Fusarium* are made up, in part, of compounds called chitin and glucan, which the enzymes break down. This process occurs over and over again in the growing tip of the fungus's microscopic, root-like structure, called a hypha.

A hypha is formed when a *Fusarium* spore—perhaps carried by wind or rain—lands on a wheat floret and germinates. The hypha's mission is to reach the developing kernel, where a storehouse of energy-rich starch compounds awaits.

In rebuilding wheat plants to make cell-wall-degrading chitinase and glucanase, the scientists intend to disrupt the hypha's orderly progress toward the food source inside the wheat plant. The strategy is to overwhelm the fungus with chitinase and glucanase that it didn't make and can't control.

Scientists have known about the role of chitinase and glucanase for years. But Okubara, Blechl, Hohn, and Berka are the first to use pieces of *Fusarium* chitinase and glucanase genes for making unique, antifungal genes. To mimic the microbe's own enzyme-making machinery as closely as possible, the researchers borrowed gene segments from a *F. graminearum* relative called *F. venenatum*. Its genes had been copied earlier into a "library"

maintained by Novo Nordisk. That made this cousin a convenient source of the needed material.

#### Tooling Wheat To Make Crucial Enzymes

The scientists already knew that engineering a wheat plant to make chitinase or glucanase was unlikely to hurt the plant itself. That's because there's little or no chitin or glucan in wheat cell walls.

Okubara, Hohn, and Marcie Moore, who is with ARS at Peoria, have extensively rebuilt some of the *F. venenatum* genes. They have, for instance, filled in missing pieces of the original *F. venenatum* chitinase-forming gene. Okubara also outfitted the genes with a custom-built promoter. This on-off switch would enable a wheat plant to better use the newly redesigned genes.

At Albany, Blechl moved the lab-built genes into wheat embryos. She started by excising the embryos from immature wheat kernels. Then she placed the embryos into petri dishes positioned in the line of fire of a gene gun, or bioblaster. Blechl ferried the genes into the embryos on gold particles propelled by the gene gun.

Later, Blechl tested the target embryos to see whether they had taken up the genes. If they had, she nurtured them into healthy greenhouse plants. Blechl then shipped seeds from the plants to colleague Ruth Dill-Macky in St. Paul, Minnesota, for a rigorous program of screening. Dill-Macky, a plant pathologist with the University of Minnesota, is part of a team that evaluates promising new wheat plants for resistance to *Fusarium* head blight. The indoor and outdoor tests pinpoint new lines that may someday replace current varieties that have been clobbered by the fungus in the past.

#### Pyramiding: A Strategy To Strengthen Defense

Preliminary results with the novel genes have been mixed. "But that's not unusual for biotech experiments," com-

ments Blechl. "Our priority is to make sure the plants are using, or expressing, the new genes to the greatest extent possible. We don't know yet if we've reached that point."

In addition, bundling these genes with others is likely a key to packing a more powerful punch against *Fusarium* head blight—an approach known as pyramiding. To fend off *Fusarium*, plants of the future might be loaded with a multiplicity of antifungal genes, for what's called multiple-gene resistance. The plant and this pathogen have co-evolved in such a way that wheat plants probably turn on many genes to survive attack.

Yet wheat harbors no single gene that enables the plants to eliminate the fungus. "Following nature's lead," explains Blechl, "we're continuing to develop an array of genes. Some are from organisms other than *Fusarium*. We think that carefully developed combinations of unique genes may provide the ultimate protection against this destructive fungus."

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*This research is part of Plant Biological and Molecular Processes, an ARS National Program (#302) described on the World Wide Web at <http://www.nps.ars.usda.gov>.*

*For more information on U.S. Patent Application Serial No. 09/649,747, "Nucleic Acid Sequences Encoding Cell-Wall Degrading Enzymes and Their Use To Engineer Resistance to Fusarium and Other Pathogens," contact Patricia A. Okubara, USDA-ARS Root Disease and Biological Control Research Unit, P.O. Box 646430, Washington State University, Pullman, WA 99164-6430; phone (509) 335-7824, fax (509) 335-7674, e-mail [pokubara@wsu.edu](mailto:pokubara@wsu.edu), or Ann E. Blechl, USDA-ARS Crop Improvement and Utilization Research Unit, Western Regional Research Center, 800 Buchanan St., Albany, CA 94710; phone (510) 559-5716, fax (510) 559-5777, e-mail [ablechl@pw.usda.gov](mailto:ablechl@pw.usda.gov). ♦*